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Homogenates derived from probiotic bacteria provide down-regulatory signals for peripheral blood mononuclear cells

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Abstract

Recently, probiotics have been under investigation for anti-inflammatory properties, especially in patients with atopic dermatitis and food allergy. Yet, the pharmacotherapeutic potential of the anti-inflammatory effect has not been documented. The present study aimed to establish the influence of non-viable, filtered bacterial homogenates on peripheral blood mononuclear cell proliferation, activation receptor expression and cytokine production. All bacterial homogenates inhibited both basal and phytohemagglutinin-stimulated peripheral blood mononuclear cell proliferation (PBMC). The suppression of PBMC proliferation by bacterial homogenates was further shown to be protein concentration-dependent. Also, the anti-proliferative potential of bacterial homogenates was comparable to the anti-proliferative effect of dexamethasone at 1 μmol/l. Moreover, *Lactobacillus* GG, *Bifidobacterium* Bb-12 and *L. acidophilus* homogenates inhibited the expression of CD25, CD69 and HLA-DR on phytohemagglutinin stimulated T lymphocytes. *Bifidobacterium* Bb-12 and *L. acidophilus* homogenates also inhibited IL2 and IL4 production. Our findings suggest that specific probiotic bacteria, or factors derived from them, may provide down-regulatory signals for peripheral blood mononuclear cell.

Keywords: Lactic acid bacteria; Probiotic; Immunomodulation; Glucocorticoid; Inflammation

1. Introduction

Probiotic bacteria are defined as '*live microbial food ingredients that are beneficial to health*' (Salminen et al., 1998). They have been shown to stabilise the gut microflora and thus improve the colonisation resistance provided by the indigenous microflora (Holzaphel, Habener, Snel, Schillinger, & Huis in't Veld, 1998). Probiotics also modulate immune responses in the gut-associated lymphoid tissue, and may thus have a strong impact on the immunological defence of the host (Havenaar & Spanhaak, 1994; Schiffrin, Brassert, Servin, Rochat, & Donnet-Hughes, 1997).

The best-characterised immunological property of probiotics is their adjuvanticity. Probiotics have been shown to enhance non-specific immune responses including phagocytic activity (Pelto, Isolauri, Lilius, Nuutila, & Salminen, 1998) and IgA-secreting cell response (Kaila et al., 1992). These functions have been associated with eradication of intestinal pathogens (Isolauri, Kaila, Mykkänen, Ling, & Salminen, 1994; Saavedra, Bauman, Oung, Perman, & Yolken, 1994). Moreover, probiotics have been demonstrated to stimulate the production of cytokines, i.e. TNF-α and IL6 (Miettinen, Vuopio-Varkila, & Varkila, 1996). There are patient groups, however, in whom immune stimulation could be undesirable, for example allergic patients with excessive immune responsiveness to ubiquitous environmental antigens and Crohn's disease patients with sustained inflammatory responsiveness to unknown stimuli, possibly including gut microflora (Duchmann et al., 1999). In these, dietary supplementation with probiotics reduced the faecal concentration of pro-inflammatory cytokines and consequently alleviated intestinal inflammation (Kalliomäki et al., 2001; Majamaa & Isolauri, 1997; Malin, Suomalainen, Saxelin, & Isolauri,

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1996; Pelto et al., 1998). Thus, probiotics also seem to possess anti-inflammatory properties, though the mechanisms of their action remain uncertain.

The present study was conducted to characterise whether the non-viable, filtered bacterial homogenates, compared to non-stimulated and phytohemagglutinin (PHA)-stimulated controls, have an effect on peripheral blood mononuclear cell (PBMC) proliferation, receptor expression and cytokine production in vitro. The comparisons between the effects of bacterial homogenates were not only assessed in relation to initial bacterial concentration but also in relation to total protein concentrations of the homogenates. To evaluate the potency of these immunomodulatory effects, parallel PBMC proliferation experiments were also carried out with an effective anti-inflammatory agent, dexamethasone.

2. Materials and methods

2.1. Bacterial strains and preparation of bacterial homogenates

Lactobacillus rhamnosus strain GG (*Lactobacillus* GG; ATCC 53103), *L. rhamnosus* strain LC-705 (*Lactobacillus* LC-705; Valio Ltd., Finland), *Lactobacillus acidophilus* (NCFB-Lb1748), *Bifidobacterium lactis* (*Bifidobacterium* Bb-12; Chr. Hansen, Denmark) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (ATCC 11842) were grown anaerobically in MRS broth at 37 °C. *Streptococcus thermophilus* T101 (Valio Ltd., Finland) and *Propionibacterium freudenreichii* ssp. *shermanii* JS (*Propionibacterium* JS; Valio Ltd., Finland) were grown anaerobically in whey permeate broth at 37 and 30 °C, respectively. Bacteria were kindly provided by Valio Ltd, R&D, Helsinki, Finland. Of the selected bacterial strains, *Lactobacillus* GG and *Bifidobacterium* Bb-12 are commercially used probiotic strains, *Lactobacillus* LC-705, *L. acidophilus* and *Propionibacterium* JS new probiotic candidate strains and *L. bulgaricus* and *Str. thermophilus* T101 commonly used dairy starters.

Precultured bacteria ($\sim 4\times10^9$ bacteria/ml) were washed three times with water and resuspended in 100 ml of water. The suspensions were then repeatedly sonicated (MSE peak sonicator, amplitude 12 microns) for 15 min to break down the cells (Pessi, Sutas, Saxelin, Kallioinen, & Isolauri, 1999). Breakdown was confirmed under light microscopy; $\sim 5\times10^8$ bacteria/ml of each strain were broken down. As the enzymes of lactobacilli have been shown to hydrolyse caseins to immunosuppressive caseins (Sutas, Hurme, & Isolauri, 1996; Sutas, Soppi et al., 1996), the proteolytic activity was assessed (Matsubara et al., 1958) and found to be non-significant. To remove viable and non-viable intact bacteria, each bacterial preparate was finally filtered (0.22 μm ∅).

The total protein concentrations of bacterial homogenates were evaluated using the Folin-Ciocalteau method. The protein yields obtained from the original bacterial concentrations were as follows: 1700 μg protein/ml for *Lactobacillus* GG, 670 μg/ml for *Lactobacillus* LC-705, 850 μg/ml for *L. acidophilus*, 960 μg/ml for *Bifidobacterium* Bb-12, 310 μg/ml for *L. bulgaricus*, 210 μg/ml for *Str. thermophilus* T101 and 70 μg/ml for *Propionibacterium* JS. In order to compare the effects of different bacterial homogenates on PBMC proliferation, bacterial homogenates were also used as diluted homogenates (protein concentration adjusted to 70 μg/ml).

2.2. Peripheral blood mononuclear cells

Human PBMC were isolated from fresh buffy coats of healthy, anonymous donors ($n=12$) (Finnish Red Cross Blood Transfusion Service, Turku, Finland) by density gradient centrifugation ($400\times g$ for 30 min) with Ficoll-Paque (Pharmacia Biotech Ltd., Uppsala, Sweden). After washing three times in Hank's balanced salt solution, the cells were counted and suspended to give a concentration of 10^6 cells/ml in Roswell Park Memorial Institute (RPMI) 1640 culture medium containing 2mM L-glutamine (Gibco Life Technologies, Paisley, Scotland), 100 U/ml penicillin (Sigma, St. Louis, MO, USA), 10 mg/ml gentamycin (Roussel Laboratories Ltd., Uxbridge, Middlesex, UK), and 10% heat-inactivated foetal calf serum.

2.3. PBMC proliferation assays

Cell cultures were prepared in triplicates in 96-well flat-bottom microtitre plates (V_{tot}/well = 150 μl). All cultures contained a total of 1×10^5 PBMC in RPMI. Negative control cultures had only PBMC whereas, in positive control culture, PBMC were stimulated by PHA (Difco Laboratories, Detroit, MI, USA) at a final concentration of 10 μg/ml (assessed as optimal concentration). Test cultures contained PBMC, a bacterial homogenate (either undiluted or diluted) or dexamethasone (Sigma, St. Louis, MO, USA) at final concentration of 0.1, 1 and 10 μmol/l and PHA. Also, negative control cultures, containing PBMC (without PHA stimulation) with a bacterial homogenate or dexamethasone, were prepared. The plates were incubated at 37 °C in 5% CO^2 for 96 h. Tritiated (^{3}H) thymidine at 0.5 μCi/well (Amersham, Buckinghamshire, UK; specific activity 123 Ci/mmol) was added to each well and the plates were incubated for a further 18 h. Cells were then harvested with an automatic cell harvester (Harvester 96, Mach IIIm, Tomtec, Wallac Ltd., Turku, Finland) and incorporation of labelled thymidine was measured in a β-counter (1450 Microbeta PLUS, Wallac Turku, Finland). The results are expressed in counts per minute (cpm; calculated by subtracting cpm of

background of β-counter from cpm of non-stimulated or PHA-induced cultures).

After the incubations, the PBMC viability of the cultures was assessed using the flow cytometer. Briefly, 1×10^5 PBMC were stained with 3.34 μM propidium iodide at room temperature (22 °C) for 5 min and analysed with a Coulter Electronics EPICS XL flow cytometer equipped with an air-cooled 488 nm argon-ion laser operating at 15 mW. Viability-% of the cultured PBMC at the end of culture periods-was constantly over 95%, indicating the functionality of the proliferation tests.

2.4. Receptor analysis

Cell cultures were prepared as in the proliferation assays except that all cultures were in duplicate in 24-well flat-bottom microtitre plates (V_{tot}/well = 300 μl). The plates were incubated at 37 °C in 5% CO^2 for 96 h. Duplicated cell cultures were then pooled and the cells washed twice with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline. After incubation, with fluorescence-labelled antibodies, the cells were analysed with a Coulter Electronics EPICS XL flow cytometer (FL, USA) (Pelto et al., 1998). FITC- and PE-labelled antibodies were purchased from Immunotech, Marseille, France (CD3, CD4, CD8, CD19, CD16, CD56, HLA-DR, CD69, CD25, and isotype controls IgG1-PE / FITC), and from Coulter Immunology, Hialeah, FL, USA (isotype control IgG2b). Results are expressed as the relative percentage of expression within the lymphocyte population.

2.5. Cytokine detection

As the previous studies have shown that probiotics may alleviate the intestinal inflammation (Kalliomäki et al., 2001; Majamaa & Isolauri, 1997; Pelto et al., 1998) and enhance IgA mediated responses (Kaila et al., 1992; Malin et al., 1996), we investigated whether these effects can be seen with the bacterial homogenates that were able to modulate activation receptor expression. Thus, we assessed the effects of homogenates of *Lactobacillus* GG, *Bifidobacterium* Bb12 and *L. acidophilus* on PHA stimulated IL2 (overall activation), IL4 (a representative of Th2 type cytokines) and TGF-β (enhance IgA responses) production.

Cell cultures were prepared as in proliferation assays. The plates were incubated at 37 °C in 5% CO^2 for 48 h. Duplicate cell cultures were then pooled and centrifuged. The supernatants were collected and stored at −70 °C. The concentrations of IL2, IL4 and TGF-β1 in the culture supernatants were determined by ELISA methods. The commercial kits were purchased from CLB, Amsterdam, Netherlands (IL4) and from Genzyme, Osny, France (IL2 and TGF-β1).

2.6. Statistical analysis

The data are presented as medians of measurements with interquartile range (IQR). The StatView 4.57 statistical program was used to analyse the data. Pairwise comparisons between non-stimulated/PHA-stimulated controls and individual test cultures, and between non-stimulated/PHA-stimulated controls or individual test cultures and cultures containing dexamethasone were carried out using the Wilcoxon signed-rank test ($P<0.05$).

3. Results

3.1. PBMC proliferation

All undiluted bacterial homogenates significantly suppressed the PHA-induced PBMC proliferation when compared to the proliferation in control cultures containing only PHA (Fig. 1A). When total protein concentration of bacterial homogenates was adjusted to 70 μg/ml, significant suppression of proliferation was found in cultures containing homogenates of *Lactobacillus* GG ($P=0.04$), *Lactobacillus* LC-705 ($P=0.03$) and *Propionibacterium* JS ($P=0.03$) (Fig. 1B). Most bacterial homogenates (non-diluted) also suppressed the PBMC proliferation at the basal conditions (without PHA stimulation) (Fig. 1C). Moreover, the suppression of PBMC proliferation was protein concentration dependent, as seen for *Lactobacillus* GG homogenate in Fig. 1D; statistical difference between A (1700 μg/ml) 1.6 (1.3–84.5) and B (850 μg/ml) 1350 (250–3100); $P=0.04$, and between B and C (70 μg/ml) 6185 (3274–10720); $P=0.02$.

Dexamethasone, at final concentrations of 0.1, 1 and 10 μmol/l, suppressed PHA-induced lymphocyte proliferation ($P=0.047$, $P=0.03$ and $P=0.03$, respectively). The effects of the homogenates of *Lactobacillus* GG, *Lactobacillus* LC-705, *L. acidophilus*, *L. bulgaricus* and *Propionibacterium* JS as undiluted homogenates did not differ from the anti-proliferative effect of dexamethasone at 10 μmol/l (Fig. 1A). At 70 μg/ml protein concentration, *Lactobacillus* GG, *Lactobacillus* LC-705, *Bifidobacterium* Bb-12, *L. acidophilus*, *L. bulgaricus* and *Propionibacterium* JS did not differ from dexamethasone at 1 μmol/l (Fig. 1B).

3.2. Lymphocyte receptor expression

Receptors of cultured lymphocytes were analysed by flow cytometry, using labelled monoclonal antibodies. PHA clearly activated the T cells, as revealed by HLA-DR, CD25 and CD69 expression (Table 1). However, PHA did not affect to the percentage of NK cells (CD3−/CD16+, CD56+), and lowered the percentage

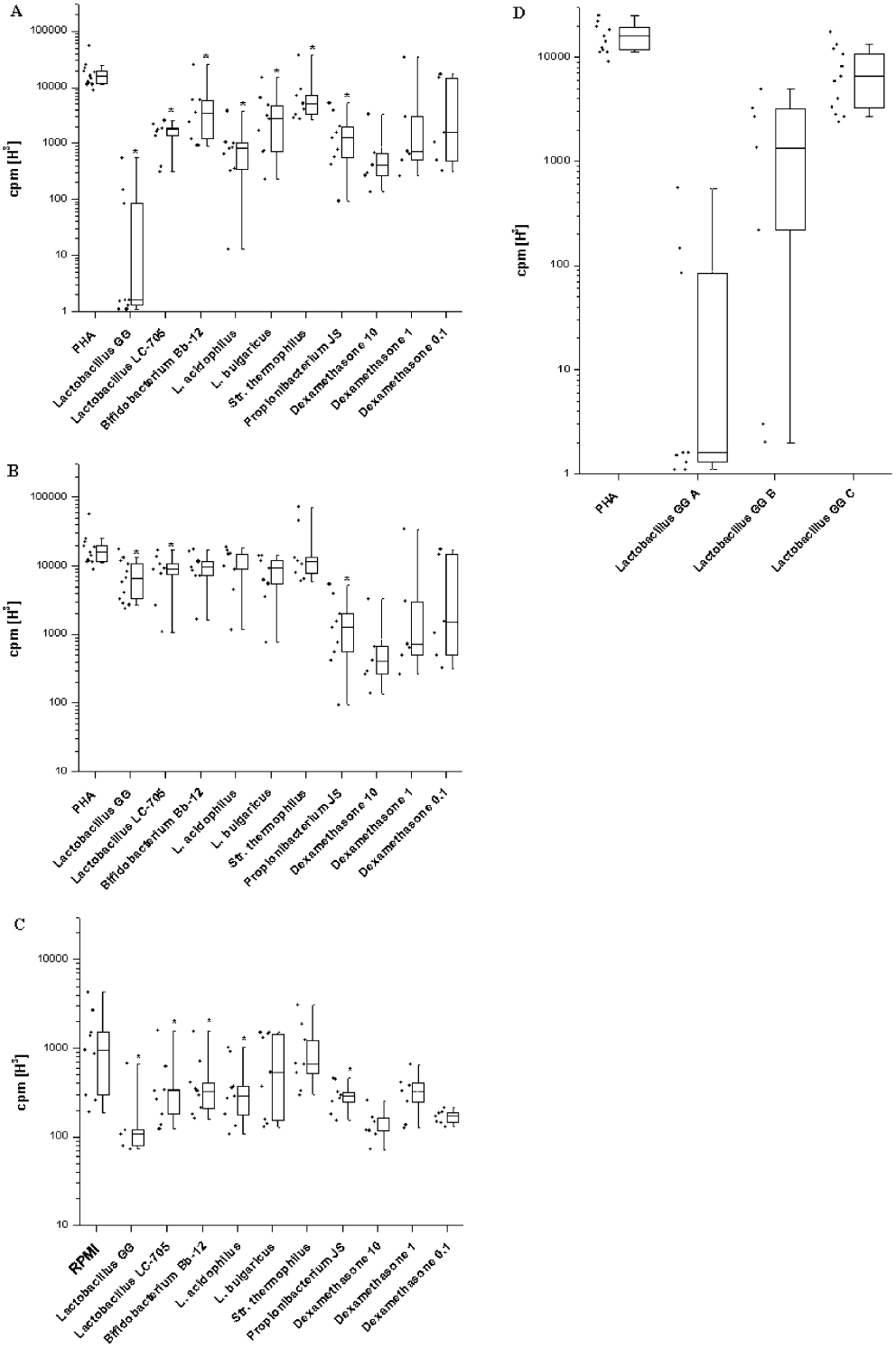

Fig. 1. The effects of bacterial homogenates and dexamethasone (at 10, 1, and 0.1 μmol/l) on lymphocyte proliferation. Proliferation was assessed in cultures containing isolated lymphocytes with either PHA only (10 μg/ml) or with both PHA and bacterial homogenate: (A) represents the lymphocyte proliferation in cultures containing undiluted bacterial homogenates (total protein concentrations of 1700, 670, 850, 960, 310, 210, and 70 μg/ml, in order of appearance): (B) shows the effects of homogenates, diluted to identical total protein concentration of 70 μg/ml; (C) shows the effect of bacterial homogenates (undiluted) on lymphocyte proliferation in basal conditions (no PHA stimulation); (D) shows the protein concentration -dependence of the antiproliferative effect of the bacterial homogenate derived from *Lactobacillus* GG (A = 1700 μg/ml, B = 850 μg/ml, and C = 70 μg/ml). The measurements are expressed as means (cpm) of triplicate analyses of individual experiments (·). The description of box plots: The central horizontal bar indicates the median, the top and bottom of the box the third and first quartiles (IQR), respectively, and the whiskers extend to 95 and 5%. Statistical differences between PHA-stimulated control cultures and cultures containing bacterial homogenates are shown as * ($P < 0.05$) (Wilcoxon signed-rank test).

Table 1
Flow cytometry was used to investigate the influence of bacterial homogenates on lymphocyte receptor expression

Receptor	Negative control ($n=12$)	Positive control ($n=12$)	Test cultures						
			Lactobacillus GG ($n=8$)	*Lactobacillus* ($n=5$)	*Bifidobacterium* ($n=12$)	*L. acidophilus* ($n=10$)	*L. bulgaricus* ($n=9$)	*S. thermophilus* ($n=5$)	*Propionibacterium* ($n=7$)
CD3+	63.0 (61.1–67.1)	66.7 (53.9–71.3)	70.4 (65.4–76.5)	64.2 (60.6–74.6)	64.9 (55.4–72.0)	65.2 (60.3–70.3)	58.7 (56.2–63.4)	59.7 (58.2–71.4)	66.8 (54.4–73.0)
CD4+	36.0 (34.6–45.0)	40.0 (31.4–45.4)	45.2 (36.5–49.6)b	43.5 (40.9–45.3)	39.8 (33.3–50.5)	45.1 (41.0–47.9)b	36.4 (26.4–40.5)	39.9 (37.2–43.6)	37.1 (31.1–50.0)
CD8+	29.3 (15.7–35.0)	33.1 (24.8–42.9)	30.5 (28.2–31.9)	22.4 (18.6–45.7)	31.3 (27.7–41.2)	25.2 (19.6–38.1)b	34.0 (28.4–38.7)	25.3 (19.5–44.5)	32.0 (20.9–39.5)
HLA-DR+	10.1 (8.2–12.6)	10.8 (7.7–12.3)	9.0 (5.3–12.7)b	5.9 (3.4–13.6)	7.0 (5.2–9.4)	7.4 (6.4–11.1)b	7.9 (5.5–12.7)	7.3 (6.1–10.1)b	5.6 (4.4–6.4)[a]
HLA-DR+/CD3+	0.7 (0.5–1.0)	6.8 (2.6–11.4)a	5.6 (1.6–9.0)b	2.0 (1.2–7.9)	3.7 (3.1–5.2)	3.8 (1.3–7.9)b	5.5 (2.5–8.9)	3.3 (2.2–6.5)	1.8 (0.6–2.8)[a]
CD25+/CD4+	7.1 (5.2–8.3)	7.4 (5.4–14.5)	4.6 (3.8–10.5)b	5.6 (5.3–8.3)	5.5 (4.1–9.1)[a]	8.4 (4.8–11.5)	4.4 (3.3–12.9)	10.2 (4.9–15.4)	4.7 (3.6–13.9)[a]
CD25+/CD8+	0.5 (0.3–1.1)	5.1 (1.6–7.2)a	0.9 (0.4–2.7)b	0.8 (0.6–6.6)b	0.7 (0.4–1.8)b[a]	1.8 (0.6–8.5)b	1.3 (0.7–8.5)	1.2 (0.5–12.3)	1.2 (0.5–2.0)
CD69+	5.2 (3.2–8.7)	28.2 (18.2–35.6)a	24.3 (12.2–32.5)	14.8 (10.0–43.3)	16.9 (11.4–27.8)[a]	28.8 (13.9–47.7)	32.2 (14.8–34.2)	17.5 (13.0–38.7)	28.6 (16.5–30.7)
CD69+/CD3+	2.1 (1.0–2.4)	14.9 (8.9–25.9)a	12.0 (5.5–21.6)b	8.1 (6.8–21.9)	10.1 (6.7–10.8)b[a]	13.2 (8.0–21.1)	15.0 (7.7–19.1)	11.5 (8.5–19.4)	9.1 (7.6–14.8)

The relative percentage of a given receptor within the lymphocyte population are expressed as median (interquartile range; IQR). Statistically significant differences ($P<0.05$; Wilcoxon signed-rank test) between negative (no stimulation) and positive controls (stimulated with PHA 10 μg/ml), and between positive control and test cultures (bacterial homogenates at the 70 μg/ml protein concentration) are represented by different letters, a and b respectively.

[a] $n=1$.

of B cells (CD19+) (data not shown). As the lowered percentage of the B cells (CD19+) is most probably an artefact caused by increased number of CD3+ cells, these results have been excluded from the interpretation of the receptor results.

Specific bacterial homogenates (at 70 μg/ml) suppressed the PHA-stimulated expression of T cell activation markers (Table 1). The overall expression of HLA-DR on lymphocytes was significantly suppressed by *Lactobacillus* GG, *L. acidophilus* and *Str. thermophilus*-derived homogenates ($P=0.02$, $P=0.02$, and $P=0.045$, respectively). *Lactobacillus* GG homogenate was able to suppress the expression of IL2 receptor (CD25) on both CD4+ ($P=0.045$) and CD8+ ($P=0.02$) T cells, whereas the homogenates of *Bifidobacterium* Bb-12, *Lactobacillus* LC-705 and *L. acidophilus* suppressed IL2 receptor expression only on cytotoxic (CD8+) T cells ($P=0.01$, $P=0.045$ and $P=0.03$, respectively). The homogenates of *Lactobacillus* GG and *Bifidobacterium* Bb-12 also suppressed the expression of CD69 on CD3+ cells ($P=0.045$ and 0.02, respectively).

Of phenotypic markers (Table 1), the percentage of CD4+ T cells was increased by *Lactobacillus* GG and *L. acidophilus* homogenates ($P=0.04$ and 0.02, respectively). As the latter also suppressed the proportion of CD8+ T cells ($P=0.03$), the CD4/CD8 T cell ratio increased from 1.4 (0.9–1.9) to 1.9 (1.3–2.6) in cultures containing *L. acidophilus* homogenate.

3.3. Cytokine production

To investigate whether bacterial homogenates mediate their suppressive effect on the PBMC proliferation and on the expression of T cell activation markers via control of the cytokine milieu, the concentrations of IL2, IL4 and TGF-β1 were determined in supernatants of test cultures containing of *Lactobacillus* GG, *Bifidobacterium* Bb-12 and *L. acidophilus*. The production of IL2 and IL4 was significantly increased by the PHA-stimulation of the cultures ($P<0.05$), whilst TGF-β production remained at the basal level despite the PHA stimulation. As shown in Fig. 2A, IL2 production differed between cultures containing undiluted bacterial homogenates [*Lactobacillus* GG 3 pg/ml (2.0–27.5); $P=0.02$, *Bifidobacterium* Bb-12 13 pg/ml (9–19); $P=0.018$ and *L. acidophilus* 25 pg/ml (23–147); $P=0.028$] and PHA-induced control cultures (65 pg/ml (58–1418)). Similarly, significantly lower IL4 concentrations in supernatants of cultures containing homogenates of *Bifidobacterium* Bb-12 [27.0 pg/ml (0.6–33.2); $P=0.008$] and *L. acidophilus* [2.2 pg/ml (0.7–31.5); $P=0.02$] in comparison to that in PHA-control [35.5 pg/ml (0.9–43.7)] (Fig. 2B). TGF-β1 production remained comparable between the test cultures and positive control culture (Fig. 2C).

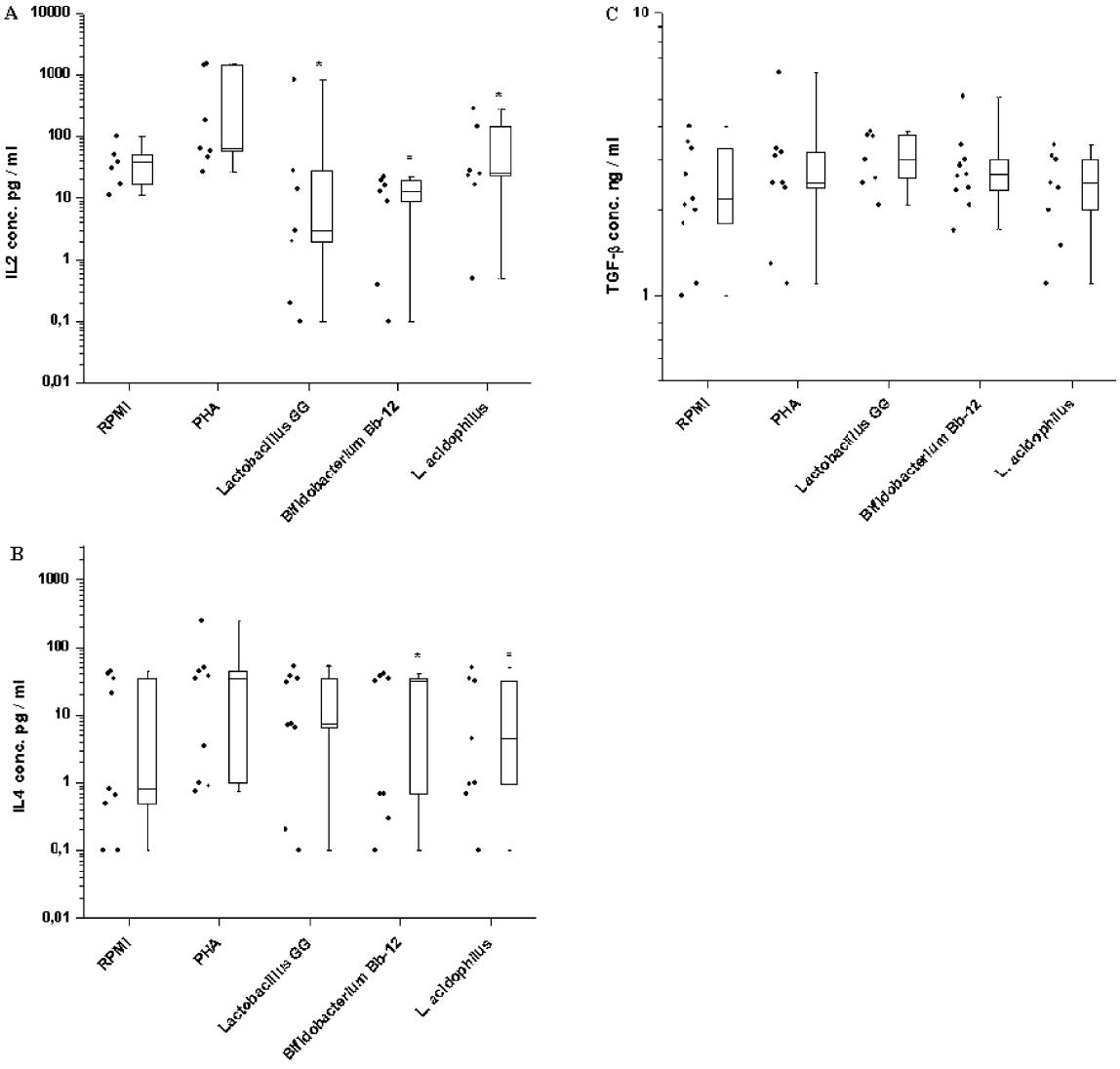

Fig. 2. (A)–(C) represent the influence of probiotic homogenates on IL2, IL4 and TGF-β production, respectively. Cytokines were determined by the ELISA method from supernatants of 48 h cultures containing isolated lymphocytes with either PHA (10 μg/ml) or both PHA and probiotic homogenates. The production of IL2 and IL4 was significantly increased by the PHA-stimulation of the cultures ($P < 0.05$), whilst TGF-β production remained at the basal level, despite the PHA stimulation. The measurements are expressed as means of duplicate analyses of individual experiments (·). The description of box plots: The central horizontal bar indicates the median, the top and bottom of the box the third and first quartiles (IQR), respectively, and the whiskers extend to 95 and 5%. Statistical differences between PHA-stimulated control cultures and cultures containing bacterial homogenates are shown as * ($P < 0.05$) (Wilcoxon signed-rank test).

4. Discussion

In contrast to pathogenic bacteria, normal members of the healthy human microflora have been assumed to regulate the state of inflammation in the gut (Wilson, Seymour, & Henderson, 1998). Probiotics, the normal constituents of the human microflora, have been demonstrated to modulate immune responses, but the immunomodulatory properties of individual strains remain elusive. The strains of *L. acidophilus* and *L. rhamnosus* have been shown to induce production of IL1, IL6, TNF-α and IFN (Kitazawa, Matsumara, Itoh, & Yamaguchi, 1992; Miettinen et al., 1996; Rangavajhyala, Shahani, Sridevi, & Srikumaran, 1997; Schiffrin, Rochat, Link-Amster, Aeschlimann, & Donnet-Hughes, 1995), whereas *L. bulgaricus* stimulated the production of IL2, IL5, IL6 (Marin et al., 1998) and produced extracellular components possessing mitogenic activity (Kitazawa et al., 1998). The genera of *Bifidobacterium* and *Streptococcus* have also been associated with adjuvant properties, and both have been shown to induce production of IL2, IL5 and IL6 (Marin et al., 1998), whereas bifidobacteria have also been associated with the induction of IL1 and TNF-α production (Miettinen et al., 1996). In contrast to these immunostimulatory activities, recent results also suggest that probiotic bacteria may possess immunosuppressive activities (Hessle, Andersson, & Wold, 2000; Kalliomäki et al., 2001; Pessi et al., 1999).

L. casei has been shown to down-regulate IL4, IL5 and antigen-induced IgE production (Shida et al., 1998), and *Lactobacillus* GG (ATCC53103) has been shown to hydrolyse bovine caseins that were able to suppress lymphocyte proliferation and down-regulate IL4 production (Sutas, Hurme, & Isolauri, 1996; Sutas, Soppi et al., 1996).

So far, most of the studies investigating the immunomodulatory effects of members of normal microflora have used either viable bacteria or cell wall extracts whereas, in the present study, we investigated the immunomodulatory effects of non-viable, filtered (0.22 μm ∅) bacterial homogenates. All bacterial homogenates tested here suppressed mitogen-induced PBMC proliferation, the most antiproliferative homogenates being *Lactobacillus* GG, *Lactobacillus* LC-705 and *Propionibacterium* JS. In general, the anti-proliferative activity of the homogenates derived from lactobacilli was protein concentration-dependent (results shown for *Lactobacillus* GG in Fig. 1D). However, *Propionibacterium* JS homogenate, with a low protein concentration, also suppressed the PBMC proliferation. Previous studies, investigating the factors involved in immunomodulation by lactobacilli, have identified these factors as lectins (carbohydrate-binding proteins) (Morata de Ambrosini, Gonzalez, Pesce de Ruiz Holgado, & Oliver, 1998) or cytoplasmic, heat-stable component(s) (Pessi et al., 1999). The substantial differences in initial protein yield, from different lactobacilli strains here, suggest that there may be multiple proteinous factors capable of suppressing the PBMC proliferation. As only ultracentrifugation would allow us to conclude that only soluble factors are present in the homogenates, we have to assume that the filtration did not remove all cell wall fragments from the homogenates. Thus the anti-proliferative activity of *Propionibacterium* JS could be governed by debris of cell wall components such as teichoic acids in the homogenate (Morata de Ambrosini et al., 1998).

The anti-proliferative effects of bacterial homogenates did not significantly differ from that of dexamethasone, a representative of glucocorticoids with known anti-proliferative potency (Baus, Andris, Dubois, Urbain, & Leo, 1996; Gillis, Grabtree, & Smith, 1979). Yet, the data presented here can not identify any specific mechanisms by which these bacterial factors may exert their activity. The exact nature of the factors involved in suppression of PBMC proliferation remains to be further investigated in study protocols utilising the interactions of these bacterial homogenates with lymphocyte membrane. Nevertheless, the proliferation results underline the pharmacotherapeutic potential of such non-viable, bacterial homogenates.

Of seven tested bacterial homogenates, *Lactobacillus* GG, *L. acidophilus* and *Bifidobacterium* Bb-12 derived homogenates inhibited the activation receptor expression. The ability of these homogenates to suppress the expression of IL2 receptor is of interest as the rate of release of soluble IL2 receptor has been shown to reflect T cell activation and inflammation in vivo (Rubin et al., 1985). *Lactobacillus* GG and *L. acidophilus* homogenates decreased the expression of HLA-DR, the finding also reported for glucocorticoids (Bootsma et al., 1998; Oeling et al., 1997). Since HLA-DR + CD3 + T cells have been demonstrated to be tolerogenic, decreasing HLA-DR expression on these cells by bacterial homogenates would decrease their tolerogenicity (Pichler & Wyss-Corey, 1994). Yet, the proliferation of the cells was suppressed, indicating that the inhibitory activity of the bacterial homogenates cannot be explained by T-cell antigen presentation-induced anergy. The inhibition of activation receptor expression observed with these three homogenates was further supported by their influence on cytokine production. *Lactobacillus* GG, *L. acidophilus* and *Bifidobacterium* Bb-12 homogenates suppressed IL2 production, whereas *Bifidobacterium* Bb-12 and *L. acidophilus* also inhibited IL4 production. However, no effect on TGF-β production was observed in our study, even though lactobacilli (*L. johnsonii*) have been shown to induce TGF-β mRNA in co-cultures with leukocytes and epithelial cells (Caco-2 cells) (Heller, Hammes, Pfeifer, Schiffrin, & Blum, 2000). Like the receptor results obtained with bacterial homogenates, the cytokine findings resembled the reported effect of glucocorticoids on peripheral cytokine production (Bessler, Straussberg, Gurary, Aloni, & Sirota, 1996; Umland et al., 1997). Yet, as no dose-response was evaluated in the receptor analysis and cytokine production-tests, one can not draw any definitive conclusion about the resemblance of the effects of bacterial homogenates and dexamethasone on PHA-stimulated receptor expression and cytokine production.

In recent years, attention has focused on the interactions between host and microbial molecules, which may determine the quality and quantity of the host immune responses (Wilson et al., 1998). Especially, there is overwhelming evidence that bacteria (mainly pathogenic bacteria) can stimulate the release of pro-inflammatory cytokines. Yet, we are not in a state of continual inflammation. The present study has shown that bacterial homogenates derived from probiotic bacteria can inhibit PBMC proliferation, activation receptor expression and production of IL2 and IL4, whereas other tested bacterial homogenates possessed only anti-proliferative properties. Despite the similarities between the effects of *Lactobacillus* GG, *L. acidophilus* and *Bifidobacterium* Bb-12 homogenates and dexamethasone, it would be speculative to refer to the physiological relevance of these results. Although, it is still unclear whether these proteinous factors are secreted by the bacteria or can cross the intestinal epithelium in order to reach

the mucosal immune system, our results indicate that (i) these proteinous factors are active even at low concentrations and (ii) are bacterial strain-specific. In concordance with recent finding of Heller and co-workers (2000), we conclude that proteinous factors derived from specific probiotic bacteria may provide down-regulatory signals for the peripheral lymphocytes.

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